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Original Article

Comparing the Effects of Diesel Oil Pollution on Forest and Industrial Soil Microbial Community

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ABSTRACT

Introduction: Diesel oil is the most used petroleum product in Iran and other countries. The majority of diesel oil is stored in underground reservoirs and Fuel stations. This product can heavily pollute the adjacent soil. Diesel oil pollution has some ecological effects on soil that disturb the composition and diversity of the microbial community. The present research aimed to investigate the effects of diesel oil pollution on two different types of soil.

Materials and Methods: To examine the effects of diesel oil on microbial communities, two different types of soil (industrial and forest types) were collected from Kerman province, Iran. Six microcosms were designed based on three microcosms existing in each type of soil, including unpolluted microcosm, polluted microcosm, and polluted microcosm with nutrients (Nitrogen and Phosphor). Some factors were assayed in each microcosm during 120 days of the experiment. These factors included total heterotrophic bacteria, total diesel oil-degrading bacteria, dehydrogenase enzyme, and diesel oil biodegradation.

Results: The quantity of diesel oil-degrading bacteria was significantly lower than heterotrophic bacteria in all soil microcosms. The quantity of diesel oil-degrading bacteria had a decrement pattern until day 60 of the experiment, but after that, these bacteria had an increment pattern. The best dehydrogenase activity between different microcosms was related to polluting microcosms with diesel oil except for farmland soil. The highest biodegradation of diesel oil in all studied soil types belonged to the industrial microcosm (95%). Statistical analysis of the results indicated a significant correlation between the most probable number quantity of heterotrophic bacteria and other assayed factors. Forest soil was significantly different from other soil types.

Conclusion: Given the obtained results of the current research, that forest soil is more sensitive to diesel oil pollution, compared to industrial soil. It is, therefore, possible to propose appropriate strategies for the bioremediation of different studied soil types.

1. Introduction

Diesel fuels are complex mixtures of saturated hydrocarbons (primarily paraffin, including n, iso, and cycloparaffins) and aromatic hydrocarbons (including naphthalene and alkylbenzene) obtained from the middle-distillate, gas-oil fraction during petroleum separation[1]. Diesel oil pollution is an environmental problem of increasing importance. Diesel oil spills from pipeline ruptures, tank failures, various production storage problems, and transportation accidents are the most frequent causes of soil and groundwater pollution[2]. Soil contamination with diesel oil negatively affects the soil ecosystem, food cycle, and microbial communities[3]. Iran is one of the world's oil-rich countries, and every year large amounts of diesel oil from the southern parts are extracted and refined in other areas. The release of diesel oil into the soil during transportation and refining causes pollution of soil and thus causes environmental contamination. Diesel oil pollution causes a decrease in the diversity of soil fauna and flora. Moreover, the spread of the contamination through rainwater cause polluted agricultural areas and

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underground water. A variety of methods have been developed to treat diesel oil contamination. While many established physical and chemical methods are efficient, they are also expensive and can cause recontamination by secondary contaminants[4]. Remediation of affected areas with the use of microorganisms can offer a cost-effective solution for restoring the ecosystem and can ensure clean groundwater supplies. Several researchers have reported microorganisms with enhanced oil-degrading abilities isolated from natural habitats historically contaminated with oil[5]. Bioremediation processes are significantly affected by the inherent capabilities of the microorganisms, their ability to overcome the bioavailability limitations in multiphase environmental scenarios (oil–water–soil), and environmental factors, such as temperature, pH, nutrients, and electron acceptor availability[6]. The current study aimed to study the response of the soil microbial community to diesel oil contamination. In this study, two types of soil, namely forest and industrial soil, were selected, and the response of each type of soil to diesel oil contamination was measured separately. To compare the effect of diesel oil contamination on these types of soil, some microbial and biochemical factors were assayed.

2. Materials and Methods

2.1. Sampling

Soil samples were collected from two different ecosystems of forest and industrial area. Forest soil was collected from the Ghaem forest at Kerman provenance, Iran, and industrial soil was collected from the Gol Gohar mine in Sirjan, Kerman provenance, Iran. Sampling was performed under sterile conditions. The first 10 cm of soil was removed, and about 3 kg of soil was poured into sterile containers. Soil samples were transported to the laboratory on ice and kept at 4ºC until further study[7].

2.2. Set-up of the microcosm systems and experimental planning

Microcosm is a small environmental laboratory designed to test conditions. Four different microcosms were performed in glass tanks (50 cm long, 10 cm deep, and 25 cm wide). Soils were sieved.

Microcosms were incubated in the dark at 25°C for 120 days. The water content of the microcosms was adjusted and maintained at 60 % of its water-holding capacity during the whole incubation period. The aerobic condition was maintained by mixing the microcosm's content every day. The soil samples were taken from each microcosm five times, including days 1, 30, 60, 90, and 120[8].

2.3. Serial dilution method

Measurements of bacterial abundance within each type of soil in the designed microcosm were performed by serial dilution procedure[9]. Heterotrophic bacteria in soils were estimated by spreading 100 µL of 10-fold diluents on Nutrient Agar medium plates and incubating at 30°C for 3 days. Diesel oil-degrading bacteria in soils were estimated by spreading 100µL of 10-fold diluents on plates of Bushnell Hass Agar medium with diesel oil and incubating at 30°C for 7 days. The results were expressed as CFU·g−1[10].

2.4. Most probable number of heterotrophic and diesel oil-degrading bacteria

Total heterotrophic and diesel oil-degrading bacteria in each microcosm were enumerated by a miniaturized most probable number (MPN) method[11]. Nutrient broth and Bushnell-Hass media were used to enumerate total heterotrophic and diesel oil-degrading bacteria, respectively. Sterile diesel oil (1 %) was used as a selective growth substrate to enumerate diesel oil-degrading bacteria. Soil samples were diluted in a saline buffer solution that contained 0.1% sodium pyrophosphate (pH 7.5). Ten-fold serial dilution was performed in microplates that were inoculated by adding 20 µL of each dilution to 1 of the 12-row wells. Diesel oil (1%) was applied to the samples as described above. The first row of each plate served as sterile control. Microplates were incubated at 20 ± 1°C for 15 days. The MPN was carried out in triplicate. The MPN counts were performed with the computer program MPN calculator[12]. All matrials were purchased from Merck Co., Germany.

2.5. Measurement of residual diesel oil

The diesel oil removal assay in the soil of microcosms was carried out by dissolving the residual diesel oil in the soil samples in dichloromethane and reading the optical density of the oil extract against blank (distill water) at 420 nm[13].

2.6. Dehydrogenase activity

Dehydrogenase activity was determined by a colorimetric method using 2, 3, 5- triphenyltetrazolium chloride as substrate. Soil samples were incubated at 37°C for 24 hours, and the reaction product, the 1,3,5-triphenylformazan, was extracted by methanol and was quantified by spectrophotometer (Shimadzu UV-160, Japan) at 488 nm[14].

2.7. Data analysis

The results of all factors examined in each microcosm during different incubation times with three replications were entered into SPSS software. The relationship between different factors was analyzed. ANOVA test was used to investigate the effect of incubation time on measured factors in soil microcosms Duncan's test was used to test the significant level of 0.05.

3. Results

3.1. The heterotrophic bacteria in soil microcosms

The quantities of heterotrophic bacteria in two studied

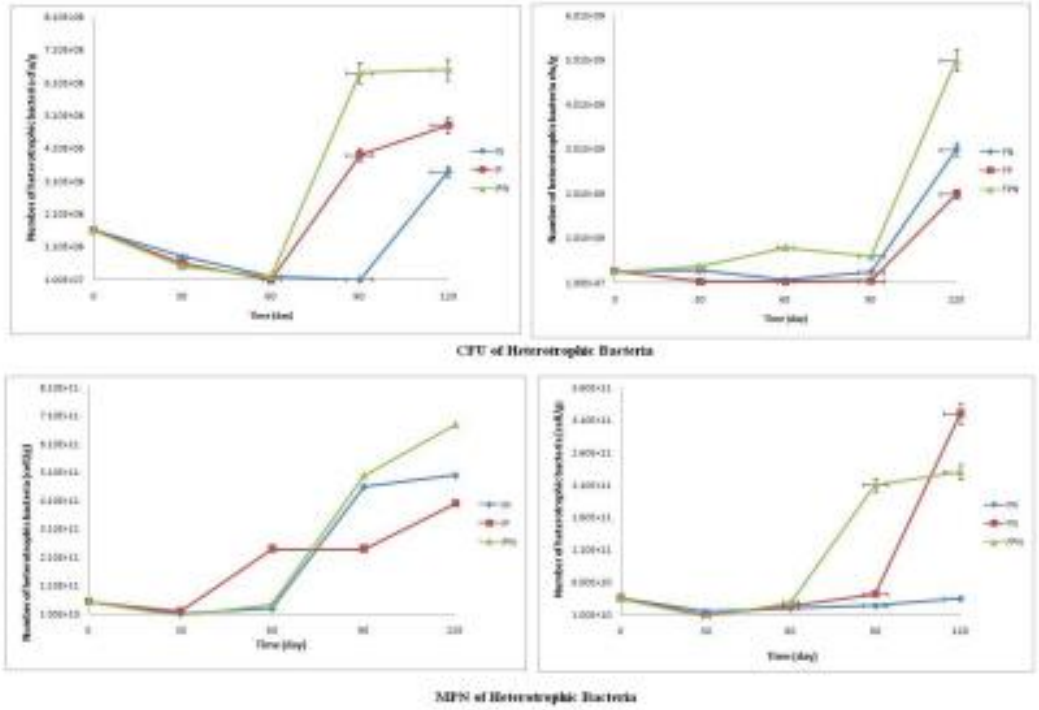

Figure 1. Enumeration of heterotrophic bacteria with serial dilution and MPN methods in studied soil microcosms

microcosms in different conditions were measured by serial dilution method. The number of heterotrophic bacteria had different patterns in these two soil types as the increase in the quantity of these bacteria in industrial soil took place on day 60 of incubation, whereas this increment for forest soil microcosms was on day 90 (Figure 1). The number of heterotrophic bacteria in forest soil (6×10^{9}) was higher than in industrial soil (7×10^{8}).

Figure 1 shows the MPN of heterotrophic bacteria in the microcosms of the current study. As can be seen, a reduction pattern of heterotrophic bacteria was observed in two polluted soil microcosms till day 60, but after that, the quantity of heterotrophic bacteria increased. After day 60, an unpolluted microcosm continues the reduction. The maximum MPN values of heterotrophic bacteria in forest and industrial soil were 3×10^{11} and 7×10^{11} (cell/g), respectively

3.2. The diesel oil-degrading bacteria in soil microcosms

Diesel oil-degrading bacteria were counted in the Bushnell Hass medium, with diesel oil as the only carbon and energy source. The results of this enumeration are illustrated in Figure 2. As indicated, the number of diesel

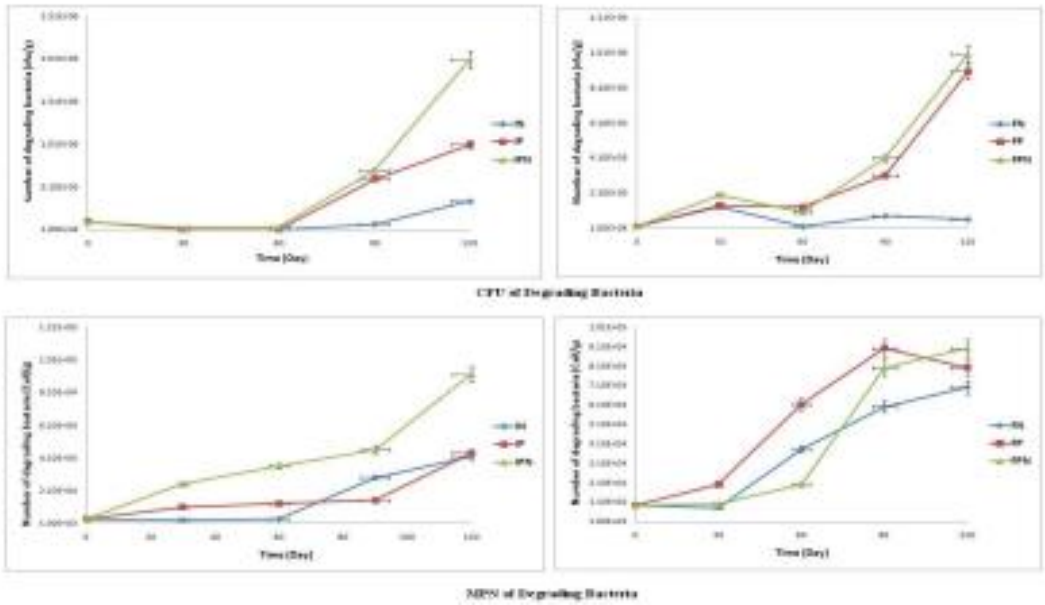

Figure 2. Quantification of diesel oil-degrading bacteria by serial dilution and most probable number methods in studied soil microcosms

oil-degrading bacteria in soil microcosms reduced until day 60 of treatment, but after that, the number of diesel oil-degrading bacteria increased in all microcosms except in uncontaminated microcosms (FN and IN). Generally, the number of diesel oil-degrading bacteria in all types of soil was dramatically less than that of heterotrophic bacteria in soli samples since diesel oil-degrading bacteria only used diesel oil, but heterotrophic bacteria could use other carbonic resources. The highest quantities of diesel oil-degrading bacteria in forest and industrial soil were 1×106 and 2×106 (CFU/g), respectively.

Diesel oil-degrading bacteria were quantified in the MPN method using diesel oil as the only carbon source and turbidity as a positive MPN indicator. As indicated in Figure 2, the quantity of diesel oil-degrading bacteria slowly increased. This pattern could be attributed to the toxic effect of diesel oil and bacteria adapted after passing the time. As can be seen in Figure 2, the number of diesel oil-degrading bacteria in contaminated microcosms with nutrients (FNP and INP), was higher than in other microcosms (FN, FP, IN, and IP). The highest MPN values of diesel oil-degrading bacteria in forest and industrial soil types were 9×103 and 8×104 (cell/g), respectively.

3.3. Dehydrogenase activity in the microcosms of the study

In industrial soil microcosms, the dehydrogenase enzyme increased in activity until day 30 of the experiment, and after that, the activity of the enzyme dramatically decreased (Figure 3). However, the opposite pattern was seen in the forest soil microcosms as the reduction in activity was seen until day 30 of incubation, followed by an increment pattern. Among three different kinds of the microcosm, polluted microcosm of oil (FN and IP) had the highest dehydrogenase enzyme activity. At least the activity of this enzyme was related to forest soil, with a value of 0.1 on day 30.

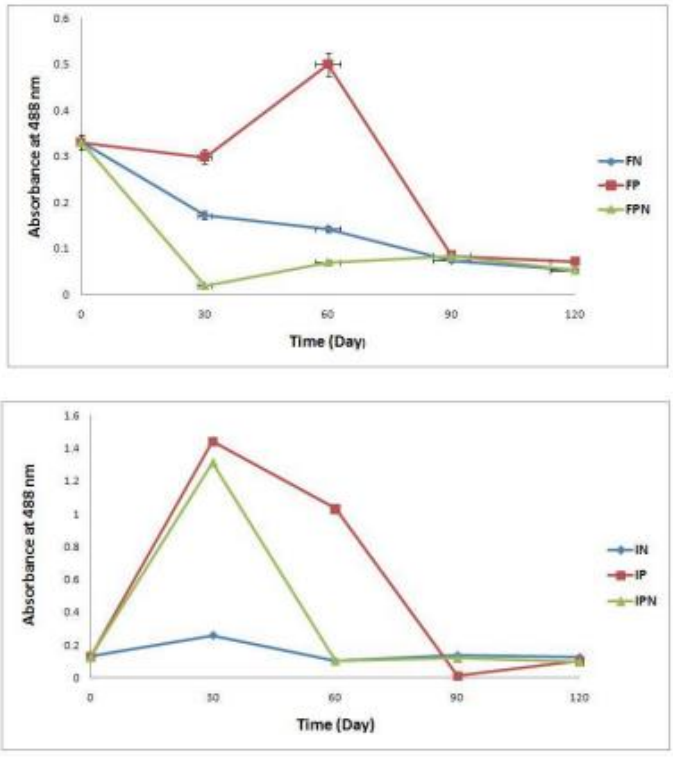

Figure 3. The activity of dehydrogenase enzyme in designed soil microcosms

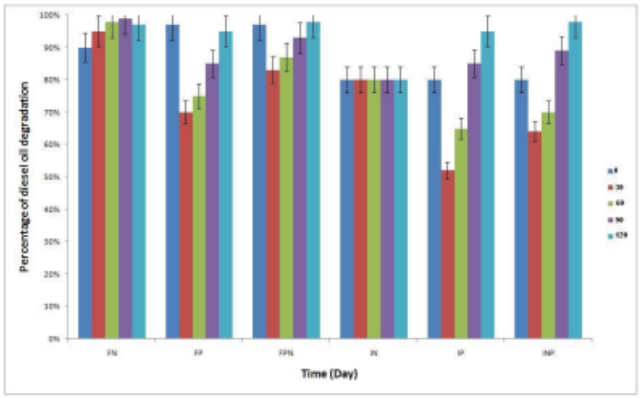

Figure 4. The percentage of diesel oil degradation in soil microcosm's during 120 days of the experiment

3.4. Diesel oil degradation in microcosms of the study

The percentage of diesel oil degradation was calculated for each soil microcosm separately. As illustrated in Figure 4, it is confirmed that by passing the incubation time, the rate of diesel oil degradation in all microcosms has an increasing pattern. Thus, the lowest degradation percentage occurred on the first day of the experiment, and the highest diesel oil degradation occurred on the last day of the experiment (day 120). However, this pattern and the degrading rate were unequal in all microcosms. The highest percentage of diesel oil degradation is related to polluted microcosms with nutrition (FNP and INP).

3.5. Statistical analysis of data

The obtained results indicated a significant relationship between the total number of heterotrophic bacteria (MPN) with other investigated factors (Table 1, $p < 0.05$). Other study factors were not significantly different ($p > 0.05$). The effect of incubation times on evaluated factors and especially the percentage of diesel oil degradation are presented in Table 2. The evaluated factors indicated significant differences on days 90 and 120 of the experiment, compared to other time

Table 1. Relationship of assay factors in mesocosms

Factor assay Duncan$_{a,b}$	N	Subset	
		1	2
Enzyme	2.89E+10	2.12E+01^{e}	
Degradation	2.89E+10	4.67E+01^{bc}	
CFU degrader	2.89E+10	3.50E+05^{dc}	
MPN degrader	2.89E+10	5.41E+04^{b}	
CFU heterotroph	2.89E+10	6.61E+08^{a}	
MPN heterotroph	2.89E+10	6.69E+08^{a}	1.73E+11
Sig.	P<0.05		

Table 2. The effect of incubation time on measured factors in soil microcosms

Duncan$_{a,b}$				
Day	**N**	**1**	**2**	**3**
0	2.04E+10^{a}	2.50E+09		
30	2.04E+10^{a}	3.96E+09		
60	2.04E+10^{a}	4.09E+09		
90	2.00E+10^{b}		3.45E+10	
120	2.00E+10^{b}			5.71E+10

spans (days 1, 30, and 60, $p < 0.05$). This result confirmed that the main changes in the microbial community took place after 60 days of oil pollution.

4. Discussion

Industrial soils have chronic contamination with petroleum products and heavy metals. The organic carbon in this soil is very high, but all soil microorganisms cannot use this carbon, and only special bacteria can use these pollutants[15]. The results of the enumeration of heterotrophic and degrading bacteria in industrial soil microcosms indicated that the number of heterotrophic bacteria in this soil was lower than in forest soil microcosms. Still, diesel oil-degrading bacteria had the highest quantity in industrial soil microcosms. These results confirmed that although the organic carbon in the industrial soil was high, the quantity of heterotrophic bacteria was low. This means that petroleum products cause the disappearance of sensitive bacteria, and only degrading bacteria were selected and prevalent in the total microbial community[16]. Regarding the response of industrial soil microcosms to diesel oil contamination, diesel oil contamination had a low effect in this microcosm, compared to forest microcosm. Two reasons confirm this interpretation. Firstly, the number of heterotrophic bacteria increased after day 60 of the experiment, whereas this increase in other microcosms took place on day 90 of incubation. Secondly, the number of diesel oil-degrading bacteria in the industrial microcosm was higher than forest microcosm. Chronic pollution that is present in industrial soil causes enrichment and selection of degrading bacteria, leading to an insignificant effect of diesel oil contamination on this soil type[14,17].

Some researchers also studied the effect of diesel oil contamination on the soil. For example, Li et al. designed 110 days experiment to understand the effect of different concentrations of diesel oil on industrial soil microcosms[18]. Their results showed that when 1000 mg/kg of diesel oil entered the soil, aerobic bacteria growth was stimulated, and there was an increase in the activity of some enzymes, such as dehydrogenase, urease, and polyphenol oxidase.

Delille and Coulon examined the simulation of diesel oil contamination on industrial soil in Belgium, showing that diesel oil-degrading bacteria dramatically increase after diesel oil contamination[17]. They conclude that after 90 days of contamination, some biological and chemical properties of the soil changed.

Forest ecosystems are very rich in organic carbon. Forest soil humus is high since the plant material and rhizosphere activity of microbes enhance the humus of the soil. In a study by Radwan et al, the highest quantity of heterotrophic bacteria reported in these soil microcosms confirmed the abundance of organic carbon in this soil, and it is used by all soil microbial communities[19].

Regarding the response of forest soil microcosms to diesel oil contamination, the number of heterotrophic bacteria dramatically decreased after diesel oil pollution. Diesel oil-degrading bacteria had lower quantities, compared to industrial soil microcosms. These results suggested that the adaptation of forest soil microbial community occurred slowly, compared to industrial soil. On the other hand, the microbial community of forest soil was more sensitive than industrial soil to diesel oil contamination[20].

Some researchers have reported the effect of hydrocarbon pollution on forest soil, and their results are in agreement with the results of the current study. For example, a study on some properties of forest soil and microbial factors in Nigeria's rainy forests after 17 years of crude oil contamination indicated that organic carbon decreased after oil contamination and the concentration of heavy metals increased[21]. The results confirmed that crude oil contamination caused a dramatic reduction in the microbial diversity of forest soil. Another study indicated a positive relationship between the remaining diesel oil concentration and quantity of organic carbon in forest soil with the activity of dehydrogenase and lipase enzyme[22].

5. Conclusion

Soil pollution by diesel oil is unavoidably present on the earth, but the critical point is accurate recognition of its negative effects and an attempt to minimize them. The results of the present research indicated higher sensitivity of forest soil to diesel oil pollution, compared to industrial soil. Moreover, the appropriate time for the soil to return to normal condition was estimated at 90 days which was significantly different from other time spans. Therefore, the obtained results of this research, based on the type of soil, can be used to recommend suitable strategies for their survival.

Declarations

Competing interests

The authors declare that they have no conflict of interest.

Authors' contribution

All authors were involved in the conceptualization and methodology of the article, data collection, data analysis and interpretation, design of the article, review, and final approval manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethical considerations

The authors checked for plagiarism and consented to the publishing of the article. The authors have also checked the article for data fabrication, double publication, and redundancy.

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